The Spermatogenesis of Lepidosiren paradoxa.

By

W. E. Agar, M.A., D.Sc.,
Zoological Laboratory, Glasgow University.

With Plates 1–5 and 1 Text-figure.

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Introduction.

The material for this work was obtained during an expedition to the swamps of the Paraguayan Chaco, undertaken

primarily for this purpose. The spot where the Lepidosirens were collected was a station of the South American Missionary Society called Naktetingma, about ninety miles west of Villa Concepcion, and about a league from the spot which Prof. Graham Kerr made his headquarters while collecting his material for working out the embryology of this fish. It is to him that I owe the whole idea of making this expedition. The expenses of the expedition were defrayed by the Government Grant Committee of the Royal Society and by the managers of the Balfour Fund of the University of of Cambridge, to both of which bodies I should like to take this opportunity of conveying my thanks. I should also like to acknowledge my indebtedness to the South American Missionary Society for the help they so cordially gave me, and especially to the little band of missionaries at Naktetingma, who most generously provided me with horses, waggons, bullocks and a home during the whole of my stay in the Chaco, and more important still, procured Indian hunters for me, and, in fact, ensured the success of the expedition.

I arrived in the Chaco in September, 1907, at the end of an unusually prolonged dry season. As is well known, Lepidosiren burrows into the mud at the bottom of the swamps when these dry up, and remains there in a torpid condition till the next rainy season sets in—normally a period of about six months. The years 1906 and 1907 were, however, unusually dry ones in the Chaco, and I was informed by the missionaries that for more than a year before my arrival there had not been enough water in the swamps to bring out the Lepidosirens—or, at any rate, to keep them out long enough to allow them to breed. Rain began to fall soon after I arrived, and by the middle of October the Lepidosirens were swimming about in the swamps. Directly they leave their burrows for the water the males start growing their peculiar vascular filaments on the pelvic fin, a sign that they are about to breed, and about the beginning of November their nests, containing eggs, began to appear in the swamps. I

started preserving testes directly I arrived, and continued doing so till February of the following year, and so got a series from the torpid dry season state through all the stages of preparation for breeding to a stage some months past the season for spawning. Although spermatogenesis seems to proceed all the year round, still the relative frequency of the different stages differs greatly in testes from the various seasons. The breeding habits and methods of hunting Lepidosiren have been described by Professor Graham Kerr.

The reason for wishing to obtain material for working out the spermatogenesis of Lepidosiren was that this form has nuclei of very great size, and in the somatic cells (as determined in Professor Graham Kerr's embryological material) the chromosomes are beautifully clear and show very pronounced size differences. In the course of this work most attention has been paid to the method by which the numerical reduction of the chromosomes takes place—owing to its importance in connecting our experimental knowledge of heredity with the structure and history of the germ-cells. Whatever may be the final outcome of the present controversy about the relative functions of the nucleus and cytoplasm in heredity, it is well established that many classes of characters are distributed alternatively to the gametes, and the only part of the hereditary substance which is visibly distributed in a like manner are the chromosomes, which are undoubtedly so distributed if there has been a previous pairing of corresponding or "homologous" ones. The observations bearing on this pairing or conjugation being at present so variously interpreted, it becomes of great interest to examine the stages in question in a form such as Lepidosiren, which besides having cytological elements of great size and distinctness, is undoubtedly closely related to the Amphibia, which also, on account of their large histological features, have been so much worked at, and have (though a single group) furnished different workers with every conceivable answer to the reduction problem.

Methods.

All the observations were made on testes fixed either with Flemming (strong formula) or corrosive-acetic. For more than a year I used ordinary paraffin sections, mostly 10 μ in thickness, and mostly stained with Heidenhain's iron-alum hæmatoxylin and eosin. Then the whole was worked through again with celloidin sections, 35–40 μ in thickness, which are thick enough to contain whole nuclei, untouched by the razor. In order to reduce to a minimum the possibility of a disarrangement of the chromatin by the processes of cutting and mounting the sections, in the great majority of cases these sections were mounted without dissolving out the celloidin. In most cases the tissue was stained in bulk with Ehrlich's hæmatoxylin before embedding, though it is possible to stain the sections after cutting. Unless the celloidin is dissolved away, however, the latter method has the disadvantage of diminishing the transparency of the sections owing to the celloidin taking up some of the stain. The sections were mounted between two coverslips instead of between a slide and a coverslip, so as to allow of the nuclei being examined from both sides. The optical apparatus employed was largely Zeiss' stereoscopic eyepiece, generally used in conjunction with an oil-immersion objective. This method is one which I found of the utmost value. Firstly, one can be certain that all the chromosomes are present and in their natural positions. Secondly, the nucleus can be examined and drawn from both sides and the chromosomes identified in the two drawings. In this way it is often possible to analyse a clump of chromosomes which otherwise could not have been separated, and the danger of overlooking a chromosome which is overlaid by another is removed. This is specially valuable in a form like Lepidosiren, where the chromosomes are large and rather numerous. The two views of the same nucleus obtained in this way are shown in Pl. 4, fig. 26, which illustrates the value of the method. Thirdly, the stereoscopic eyepiece is an immense help (as a supplement to

eyepieces of a higher power) in unravelling a complicated spireme or a nucleus full of chromosomes.

The Number of Chromosomes.

The somatic number of chromosomes is thirty-eight. Both this and the reduced number, nineteen, have been counted over and over again in the meiotic nuclei. Murray gave the somatic number as probably thirty-six, which is as near the right number as could be expected to be arrived at from the somatic mitoses with their long chromosomes.

The Germinal Epithelium.

The testes are composed of numerous convoluted tubules which in a transverse section appear cut through in every direction. These tubules are lined with the germinal epithelium, which, in an adult breeding Lepidosiren, consists of spermatogonia of all orders, spermatocytes and spermatids, mixed up together with very little arrangement. There is no definite layering of the cells of the successive generations, but on the whole those of the later orders are nearer the lumen of the tubules than those of the earlier ones. Still, it is impossible in most cases to say from its position in the germinal epithelium to what generation a given cell belongs. Also all the tubules throughout the whole length of any testis present the same stages. Nevertheless the seriation has not been a matter of much difficulty. The testis tubules branch and wind about in an extremely complicated way, and at the blind end of the branches one always finds a solid, though generally very small, mass of spermatogonia. I also got one immature male, just after the beginning of the rainy season but before the fish had come out of the ground, in which the testis consists almost entirely of spermatogonia with very few primary spermatocytes and one fully formed spermatozoon to every score or two of sections. Another useful specimen was an adult male preserved at the same season in which the testes

consist largely of spermatogonia of the earlier generations, arranged in many tubules as a definite lining epithelium.

With the help of these three specimens, and of the little clumps of spermatogonia at the blind ends of the testis tubules of breeding males, it was easy to learn to recognise, both in the "resting" and dividing states, the different cells of which the germinal epithelium is composed. The nuclei of these cells bear a close resemblance to those found in the salamander. Meves' description of the different generations of cells in the testis of this animal could be applied closely to Lepidosiren.

The spermatogonia are often divided into two classes—primary and secondary—but it is doubtful whether such a sharp distinction can be drawn in Lepidosiren. It is safer to speak of earlier and later generations of spermatogonia. Among the earlier generations are found many nuclei corresponding exactly with those described as primary spermatogonia by Meves. The chromatin in these, as in all the spermatogonia, is in the form of large irregular blocks connected by finer strands. What is particularly characteristic about these primary spermatogonia, however, is, that the chromatin is often obscured or almost entirely concealed by a diffusely staining substance filling the whole nucleus (Pl. 1, fig. 1). Meves notes this as a characteristic of the primary spermatogonia of the salamander, and considers that the substance is a precipitate produced by the action of the fixative, since it is not found in the nuclei of the same cells in the centre of the sections. The impression gained from a study of Lepidosiren is, that the substance is most dense in nuclei which are undergoing a prolonged rest, and least abundant or altogether absent in those in a period of active multiplication. At any rate, as soon as the prophase has definitely set in the nuclei are quite clear except for the sharply defined chromatin.

The nuclei of these earlier generations of spermatogonia are frequently polymorphic—a characteristic of this class of nucleus in many animals. Pl. 1, fig. 1 shows a moderately

lobed nucleus. Sometimes appearances strongly suggestive of amitosis are observed, but I have never seen any sign of accompanying cell division, and I have little doubt that direct division does not occur. In the cleaving egg of Lepidosiren the nuclei of the blastomeres are often strongly lobed, but when the prophase sets in these lobes are drawn in and the nucleus becomes more and more spherical in outline. Although I have not seen this occurring in the case of the polymorphic spermatogonia (probably because the rounding off takes place before the prophasic changes are apparent), it seems most probable that this happens also in the case of these nuclei. The polymorphic nuclei are always especially rich in the diffuse substance already mentioned. The later generations of spermatogonia differ from the earlier ones chiefly in size, both of nucleus and cell-body. At a certain stage in its life-history the spermatogonium seems to pass into a period of more rapid division, and it is this rather ill-defined point that may be taken as separating the primary from the secondary spermatogonia. In consequence of the successive rapid divisions the size of the nucleus decreases from about 30 μ to about 17 μ or less in diameter. Fig. 2 shows a nucleus belonging to this latter period. Its structure is the same as before, but there is none of the darkly staining ground substance shown in Pl. 1, fig. 1. I have never found one of these smaller nuclei polymorphic.

Although it should logically come at this point, it will be best to delay the description of the spermatogonial mitoses till after the meiotic (maturation) divisions have been described, as this will bring out more clearly the significant differences between the meiotic and pre-meiotic prophases.

The last generation of small spermatogonia gives rise to the primary spermatocytes, which increase during the growth period till they are larger than the largest spermatogonium, being frequently over 30 μ in diameter at the time that they are preparing to enter the meiotic prophase. The resting nucleus of the primary spermatocyte at the end of the growth period has a very characteristic structure, very

distinct from that of the spermatogonia. The chromatin is in the form of an extremely fine meshwork, without any large blocks at all (Pl. 1, fig. 8). There is a single large nucleolus (not visible in fig. 8, but shown in fig. 9), which is very characteristic of this stage.

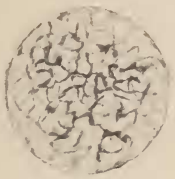
The Meiotic (Maturation) Divisions.

Nomenclature.—For the various stages of the prophase I have used the nomenclature now generally adopted, based on that proposed by von Winiwarter in his work on mammalian oögenesis. The stages run: leptonema, zygonema, pachynema, strepsinema or diplonema (adjectives leptotene, zygotene, etc.), and diakinesis. In pachynema the chromatin threads are arranged in the characteristic bouquet grouping. The one-sided contraction of the chromatin may occur at any of the above stages—in Lepidosiren it begins in the strepsitene stage. In order to avoid the confusion caused by the word "synapsis," I have here employed Mr. Clung's term synizesis for the visible clumping together of the chromatin, and Häcker's word syndesis for the conjugation of the chromosomes.

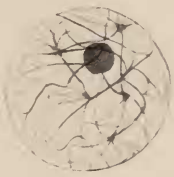
The most important stages in the maturation divisions are shown in the text-figure.

First Meiotic Division.—The first indication of the approaching mitosis is that the dense reticulum of the resting primary spermatocyte gradually gives way to a condition in which certain principal threads can be recognised and followed for a considerable distance, and this gradually changes into the leptotene stage in which all the chromatin is in the form of very long fine threads, which twist about and cross one another in a most complicated way. A nucleus in this stage is shown in Pl. 1, fig. 9. The fine threads are so numerous and involved that, in order to avoid confusion, I have only figured those threads which lie immediately below the nuclear membrane in one hemisphere. In reality the whole volume of the nucleus is filled with the tangle of threads. It should

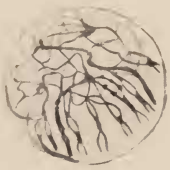
TEXT-FIG.



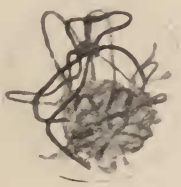
A
Resting Spermatocyte I.



B
Leptonema.



C
Lepto-zygonema.

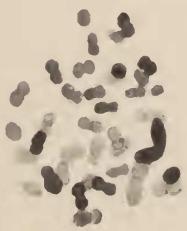


F
Advanced Synizesis, Rings beginning to break up.

E
Strepsinema, and beginning of Synizesis.

D
Zygo-pachynema.

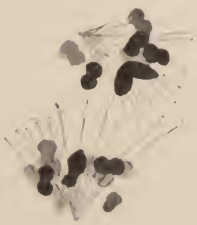
G
Bivalent rings breaking up into their constituents.



H
Disappearance of Nuclear membrane. Somatic number of transversely constricted univalents.

I
Second pairing of the chromosomes.

L
Metaphase II.



K
Anaphase I
Spindles rotating for the second division.

J
Second pairing complete. Definitive bivalents being placed on the spindle.

TEXT—FIG. GENERAL SCHEME OF MEIOSIS IN THE MALE LEPIDOSIREN.

be specially noticed that at present there is no parallelism of threads beyond what is certainly accountable for by pure accident.

The next advance is that at one spot of the nucleus (the "pole") these threads become arranged in a parallel manner for a short part of their course, and then they immediately begin to fuse together in pairs. This is, of course, the beginning of the zygotene stage, the interpretation of which has been the matter of so much controversy. As is well known, one set of workers maintains that each member of the pairs of fusing threads is a complete chromosome, and that the stage is the one in which numerical reduction of the chromosomes takes place (syndesis), while others hold that each thread is only equivalent to the daughter halves of a precociously and temporarily split chromosome. In describing the subsequent maturation processes it is necessary, in order to avoid great circumlocution, to use terms which presume the truth of one or other of these two views, and as I believe that in Lepidosiren the evidence is on the whole in favour of the first view, I have used expressions in accordance with it, such as conjugants for fusing leptotene threads, etc.

The earliest of the three zygotene stages figured is Pl. 1, fig. 10. The pairs of leptotene threads have in no case fused over more than a small portion of their length. The thick threads formed by the fusion of two leptotene filaments are, of course, the beginnings of the pachytene threads. In some cases the fusing threads can be seen to be twisted round one another in a complicated way. Towards the other side of the nucleus the conjugating threads diverge, and can be traced into the general leptotene complex, but the individual threads cannot be traced far owing to their complicated courses, and to a certain stickiness which appears to make them adhere together where they cross one another. As usual the threads have their free ends orientated in the same direction, i. e. towards the polar end of the nucleus, and it is this pole that fusion is beginning at, and spreading away from. From analogy with other forms we may probably

safely assume that the chromatin threads are all pointed to that part of the cytoplasm in which is embedded the centrosome, or, at any rate, the kinetic centre of the cell, though I could not be sure of this structure at this stage in Lepidosiren.

Pl. 2, fig. 12, shows a zygotene stage (rather more advanced than Pl. 1, figs. 10 and 11), seen from the pole. In this nucleus the free ends of the fusing pairs are many of them embedded in a rather faintly staining coagulum, a condition which we meet with again, though not so pronounced, in Pl. 2, fig. 14.

In Pl. 1, figs. 10 and 11, it is well seen how widely diverging the conjugating leptotene threads are as they pass away from the nuclear pole—a condition hard to reconcile with the view that we are here dealing with a re-fusion of temporarily separated daughter-halves of the chromosomes. This view meets with still further difficulties when we trace back the fusing threads of the zygotene stage to the leptotene threads of the preceding stage, which, as we saw, are not arranged in any paired way at all.

Some authors have adduced in support of the theory of parallel conjugation the fact that no threads of an intermediate thickness between leptotene and pachytene filaments can be found. Others, having found that in the forms studied by them such intermediates are present, have considered this fact as strong evidence against the existence of this mode of conjugation. In Lepidosiren it is certainly true that it is not always possible to tell whether a given chromatin thread is of the leptotene or pachytene order, but this is no evidence against the view of the formation of the pachytene threads by fusion of pairs of leptotene filaments. From the moment of their first appearance the threads are continually shortening and thickening, and it is probable that conjugation does not always take place at precisely the same stage of contraction. Moreover, the intertwining and stickiness of the threads seems to cause them to contract unevenly, so that the same filament may be thicker in one part where its

condensation has been unrestricted than in another where its entanglement with other threads acting as relatively fixed points has kept it stretched thin.

The direct evidence for this mode of syndesis is to be found in the zygotene nuclei, such as Pl. 1, fig. 10, for example, where there is no doubt that the threads in the unorientated part of the nucleus are of the same order of thickness as the incompletely fused moieties of the pachytene threads.

As a result of their work on Tomopteris, Salamandra, Spinax, and Myxine, the Schreiners think that they can formulate the hypothesis that chromatin granules are present in the same number in homologous chromosomes, and that conjugation consists essentially in the fusion of these granules in pairs. The evidence presented by Lepidosiren is negative in this respect. As a rule the conjugating threads do not present any granulation, this making its appearance in the pachytene stage (see Pl. 2, figs. 13, 14, and 15). In Pl. 1, fig. 10, it is true we do see one pair of threads each with three large chromatin granules which are about to fuse together. This condition is, however, not the rule but the exception. It may be that the general absence of chromatin beads in the conjugating threads is due to the fact that in Lepidosiren conjugation takes place very early, i.e. while the threads are still extremely long and thin. For it is possible that the granules are really present but so drawn out as not to be readily demarcated from one another. Their appearance in the later stages of the pachytene nucleus would then be explained by the continuous contraction of the threads, whereby the granules are enabled to assume their spherical shape.

In Pl. 2, figs. 13 and 14, conjugation has proceeded farther and the arrangement of the pachytene threads in the characteristic bouquet is becoming apparent. Fig. 13 is drawn from the side, fig. 14 from the pole. In fig. 13 many of the threads show no sign of doubleness, but in some of them their leptotene constituents diverge and run separately for a considerable distance. In the nucleus figured in fig. 14

fusion seems to be completed, at any rate over the polar hemisphere. Each pachytene thread now appears as a single band, with a pronounced beaded appearance.

It is still impossible to count the number of complete threads in the nucleus owing to their large number and great length, and in most nuclei the onset of synizesis at about this stage renders it impossible to do so until the diakinetic chromosomes make their appearance. I found one nucleus, however, figured in Pl. 2, fig. 15, which has obviously remained in the bouquet stage for a longer period than usual, and consequently the shortening of the chromosomes (which proceeds continuously during the whole of the prophase till the disappearance of the nuclear membrane) allows of an approximate count of the pachytene loops being made. This nucleus, which is intact in a 40 μ celloidin section, was drawn from both sides and the number of free ends directed towards the pole was counted. In the figure only the superficial chromatin of the upper half of the nucleus is shown, but owing to the pole being slightly tilted up far more than half the total number of free ends are shown. From the examination of this nucleus from both sides, I can say with certainty that there are rather less than thirty-eight free ends directed towards the pole. As the great majority of the chromosomes, as in the bouquet stages of other forms, are in the form of horse-shoe-shaped loops with two free ends, it is quite certain that the loops are in the reduced number, that is nineteen.

This is in accordance with what we know of the bouquet stage in other forms. The loops have been found to be present in half the somatic number in all those species in which, owing to the small number of the chromosomes, it has been possible to count them at this stage. Grégoire (1910) gives the following list of such forms: Amphibia (all since Flemming up to the most recent workers), Tomopteris (Schreiner), Cyclops strenuus (Lerat), Pedicellina (Dublin), Ophryotrocha (Grégoire and Deton, Schreiner), Thysanozoon (Deton), Ascaris (Griggs), Zoogonus (Grégoire). To this list, which could certainly be much

extended, I might add as the most recent examples, Planaria (Arnold), Dicrocoelium (Dingler).

One of the objections which Meves (1907) urges against interpreting what takes place in the zygotene stage as a fusion of originally separated threads, is that the linin bridges which connect up all the threads (not merely the members of the conjugating pairs) must prevent the necessary movements. In Lepidosiren, however, it is a matter of observation to determine that threads, at one time separate, do move together and fuse in spite of the existence of linin bridges. Whether we must suppose that the linin connections are sufficiently elastic to allow of the necessary movement, or whether Grégoire is right in supposing that the approximation of the threads is accompanied by a breaking of the connections, which may be formed again afterwards, is a point which must be left undecided so far as Lepidosiren is concerned, though the former view seems to be indicated.

Following immediately after the bouquet is the strepsitene or diplotene stage, in which the conjugants which were temporarily united in the pachytene loops separate again. In Lepidosiren this stage is complicated by the fact that a very pronounced synizesis (synapsis of some authors) now sets in. As it is now certain that synizesis does not always coincide with syndesis (it does not for instance in Lepidosiren) the discussion as to whether the contraction is or is not an artefact has lost the interest which it had when it was supposed by many workers to be the necessary concomitant of the conjugation of the chromosomes. In my material it always occurs between the bouquet stage and the appearance of the definite chromosomes, both in testes fixed with Flemming and with corrosive-acetic, and equally in all parts of the section. The stage in which it occurs is therefore that in which the individual members of the tangled mass of chromatin threads undergo a very pronounced contraction, and it seems that this fact must set up a condition of stress within the nucleus, the visible result of which is the clumping together of the threads at the side of the nucleus away from

that in which the attachment of the chromosomes to the nuclear membrane first gave way. Whether this clumping together occurred during life or whether it needed the additional strain of the fixation to bring it about is probably an unimportant matter.

The fact that the onset of synizesis coincides with the entrance of the nucleus into strepsinema causes the details of the latter to be less diagrammatic than in many other forms, e.g. Tomopteris, Salamandra (Schreiner), Dicrocoelium (Dingler). The separation of the conjugants also takes place very quickly along their whole length, except (for a time) at their ends, so that the pachytene threads are rapidly converted into very long rings. This is shown in Pl. 2, fig. 16, which is a polar view of a strepsitene nucleus. Instead of the free ends of the pachytene threads we find that each of them has split and forms a long ring, the greater part of which is hidden in the synizetic mass below. In the case of most of the threads the conjugants have separated completely, so far as can be seen, except at their ends, but some of them can be seen in the act of separating, being still fused together in places. The shortening and thickening of the threads has, of course, been proceeding steadily since the first onset of the prophase, so that the separating conjugants are far thicker than they were at the time when they fused in zygonema. The chromosomes have lost the beaded appearance they possessed in the bouquet stage.

From the study of the preceding and following stages as well as by analogy with other forms we know that the rings are present in the reduced number. In the stages immediately succeeding strepsinema in Lepidosiren a very curious process takes place. The rings break through first at one joint (i.e. point of attachment of conjugants) to form open loops, and then at the other to form perfectly separate chromosomes. In late diakinesis we thus get the somatic number of chromosomes present, which show no signs whatever of having been previously united.

Figs. 17–24 illustrate these stages. In the interval covered

by these figures we are concerned with four separate processes, which do not always keep exact pace with one another. These processes are: the increase in intensity of synizesis up to a maximum, and then its gradual loosening out; the shortening and thickening of the chromosomes; the complete separation of the ex-conjugants already mentioned; and the development of a transverse constriction across each (univalent) chromosome.

In Pl. 2, fig. 17, synizesis is very pronounced, and the chromosomes have contracted considerably since the stage shown in Pl. 2, fig. 16. Most of them are probably still in the form of rings, though the dense mass in the centre does not allow of any of them being followed out completely. There is, however, one free end which must have been formed by the breaking open of a ring. At *s* is seen another ring in the act of breaking through at the point of junction of the conjugants.

The completion of the process of separation of the conjugants can be followed in figs. 18–24. These are all in thick celloidin sections (with the exception of fig. 24), and untouched by the razor (except figs. 21 and 24). The dissociation of the conjugants, therefore, cannot possibly be ascribed to dislocation by the razor.

In Pl. 2, fig. 18, owing to the still considerable length of the chromosomes, and to the fact that synizesis is not quite resolved, it is not possible to determine the limits of all the chromosomes. Among those which can clearly be made out there is one complete bivalent ring (*b1*), three bivalents in which the constituents are united by their ends only (*b2*–*b4*), two pairs of univalents, still, however, united by a thin thread (*s1*, *s2*), and a number of free univalents. Thus we get here every stage in the breaking apart of the rings. In the still complete ring *b1* it can be seen that the conjugants are in the act of tearing apart at one junction, while the attachment at the other one is not intimate.

In Pl. 2, fig. 19, the synizesis is still at its height, and only a few of the chromosomes can be determined. Among the free

ones, however, are one bivalent ring (*b*), one pair of recently separated univalents (*s*), and a number of free univalents. In the univalents the transverse constriction is beginning to develop.

In Pl. 3, fig. 20 A synizesis has almost broken up, but there are still some chromosomes hidden away among the main clump. At least thirty free ones can be made out, and of these one is a bivalent ring (*b*). One pair of very long chromosomes is also seen still united by a thin thread (*s*). Owing to the fact that the transverse constrictions are beginning to develop, it is not in every case easy to determine the valency of the chromosomes.

The pair of long chromosomes (*l*), which are seen united by a thread only at *s* in this figure, can be traced from now onwards to the end of the second maturation division. They can also be seen in the spermatogonial mitoses (Pl. 1, fig. 7) and probably also in the somatic nuclei.

In Pl. 3, fig. 21 synizesis has quite broken up and the separation of the ex-conjugants is complete. On the other hand the shortening and transverse constriction of the chromosomes has not proceeded so far as in the preceding figure. The somatic number of univalents can be easily seen. The wide separation of the "homologous" chromosomes is well seen in the case of the large pair; it will be noticed that several chromosomes are lying between them. Although this nucleus, unlike the others figured of this stage, has been grazed by the razor, the wide separation of this pair of chromosomes cannot be due to dislocation by the knife, as they lie towards the untouched surface of the nucleus, and the section is a celloidin one, mounted without dissolving out the celloidin.

The nucleus shown in Pl. 3, fig. 22, is further advanced in that the transverse constriction of the univalents is much more marked, but the dissociation of the ex-conjugants is not so complete. The chromosomes can be easily counted, and we find nine bivalents and twenty univalents (= 38 uni-

valents). Of the bivalents four are closed rings[1] (*b*1–*b*4), three of the others have their constituents separated except at one point of contact (*b*5–*b*7), and in two of them the ex-conjugants have almost completed their separation (*s*1, *s*2).

In Pl. 3, fig. 23, the fact that the transverse constrictions are only incipient in many of the chromosomes makes it difficult to value them, but it is certain that there is a mixture of uni- and bi-valents, the former predominating.

At the time that the nuclear membrane disappears separation of the ex-conjugants is generally complete, and we have thirty-eight quite free univalent, transversely constricted chromosomes (Pl. 3, fig. 24). As regards this constriction, it may be said at once that it does not represent a future division plane of the chromosome (see pp. 22, 29).

As the individual chromosomes become free from the synizetic mass they tend to become arranged close under the nuclear membrane, leaving the centre of the nuclear space empty. This condition of peripheral distribution of the chromosomes shortly before the disappearance of the membrane is a widely distributed phenomenon, but in the case of the meiotic prophase it is usual to find them in the reduced instead of the somatic number. Indeed, this is often the stage at which the peculiar shapes of the bivalents (rings, crosses, tetrads, etc.) is best made out.

During the period covered by the last few figures the nucleolus, which was such a conspicuous object until it was hidden in synizesis, gradually loses its staining capacity, becomes irregular in outline, and disappears.

Just before the dissolution of the nuclear membrane the diameters of the nuclei vary round an average of about 35 μ.

When the membrane disappears we get the condition shown in Pl. 3, fig. 24. At this stage no achromatic figure can be seen

[1] It must be remembered that this nucleus, like all the others about this stage, was examined and drawn from both sides. In the case of the rings *b*1–*b*3, which are seen in end view, it is difficult to give proof of their ring nature in the figure, though this is at once apparent on examination of the same chromosome from both surfaces.

with certainty, but soon this makes its appearance, and at the same time the chromosomes begin to pair again to form the rings, some modification of which nearly all of them assume on the equatorial plate. This secondary pairing of the chromosomes, which takes place in exactly the reverse way to the previous separation, is illustrated by Pl. 4, figs. 25–28, which also show the mode of development of the spindle figure. It will hardly be necessary to say that the second pairing unites the same chromosomes that conjugated in the first instance; this is shown clearly by the equality of the members of each pair.[1]

In Pl. 4, fig. 25 the spindle axes are only separated through an angle of about 45°. Of the thirty-eight chromosomes, twenty-six are still separate univalents, but the remaining twelve have joined to form six bivalents. In the case of three of them (*b*1–*b*3) pairing is complete, and they form closed rings. In the case of the other three (*b*4–*b*6) they have at present united at one end only, forming bivalent quadripartite bands. Presently the open bivalents so formed will bend round and form closed rings also.

This nucleus, like those in Pl. 4, figs. 26 and 27, is in a thick celloidin section, and was carefully drawn from both sides to verify all the chromosomes. In fig. 26 are shown the two drawings of another nucleus obtained in this way. In both figures all the chromosomes are shown, but those in the upper optical section are shaded, and those in the lower half shown in outline only.

In this nucleus, although the spindles are further separated, pairing has not proceeded so far as in Pl. 4, fig. 25. The thirty-eight chromosomes can be plainly counted, and of these only two have completely joined to form a ring (*b*). There are four couples in an early stage of pairing—Nos. 1

[1] The equality is only approximate, but the differences in size are slight enough to be accounted for by the condensation having proceeded at slightly unequal rates and by slight irregularities in outline. At any rate, in the case of the large pair, these are so much greater than any others that there is generally no possibility of mistaking them.

and 3, 5 and 6, 7 and 8, 9 and 10. None of these are as yet in actual contact, but they are in each case united by one or two linin threads. With one exception none of the other univalents are connected by visible bridges. It is an interesting question whether the linin thread which appears between two chromosomes shortly before they pair is a new formation, or only a becoming visible of a previously existing structure. Everything points to the connection being a new formation. In Pl. 3, fig. 24, no connections are visible, and in order to see whether they could be brought into view by over-staining the cover-slip was taken off and the section densely re-stained with Heidenhain's hæmatoxylin. This process, however, did not result in showing any linin connections between any of the chromosomes. The frequent wide separation of the two members of a pair is also against their being really connected all the time by an invisible thread. The linin bridges seem to act as contractible fibres to pull the chromosomes together. Heidenhain considers linin to be a specifically contractile substance. In this figure Nos. 2 and 4 are probably the large chromosomes still unpaired.

In Pl. 4, fig. 27, the spindle apices have nearly reached opposite poles, and pairing is almost complete. There are in fact only two unpaired univalents ($u1$, $u2$). Of the bivalents fifteen are completed rings or "tetrad" modifications of rings, and three are joined by one point of contact only. One of these (l) is the pair of large chromosomes.

In Pl. 4, fig. 28, the spindle is practically ready, and pairing is complete. Only a few of the chromosomes are figured to show the characteristic shapes of the bivalents and their great difference in size. The large pair, now a complete ring, is shown at l. Some of the gemini are typical "tetrads," the appearance being due to the pronounced transverse constrictions and close lateral approximation of the two univalents composing them.

As will be seen by comparing Pl. 4, figs. 25 and 26, the process of pairing does not always proceed exactly *pari passu* with the separation of the spindles. Indeed, sometimes

nuclei are found in which pairing is complete by the time the spindles have separated through a very small angle only. In general, however, the less the time that has elapsed since the disappearance of the nuclear membrane the larger the proportion of univalents, and the nearer the spindle is to completion the greater the number of bivalents.

The following is a summary of the behaviour of the chromosomes up to this stage. They appear out of the resting nucleus as the leptotene threads. These are intimately united in pairs along their whole length in zygonema. In strepsinema they separate again, but for a time remain attached to each other by their ends to form long rings. These rings open, first at one point of contact of the conjugants, then at the other, and the somatic number of univalent chromosomes is again obtained. Each univalent as it shortens becomes transversely constricted. After the dissolution of the nuclear membrane and appearance of the achromatic figure the chromosomes that were previously united pair again. This pairing takes place by a process exactly the reverse of the previous separation. First, a linin thread appears connecting a pair of corresponding chromosomes; then these come into contact by one end to form a rod-shaped (quadripartite) bivalent; then the rod bends round on itself to form a closed ring or modification of a ring.

In the metaphase of the first meiotic division one member of each bivalent goes to one daughter-nucleus, the other to the other.

The chromosomes on the equatorial plate present the same shapes as shown in Pl. 4, fig. 28. In Pl. 4, fig. 29, is seen a polar view of this stage, to show the nineteen gemini. Pl. 4, fig. 30 shows an early anaphase, to show the mode of separation of the chromosomes. The large pair is well seen. None of the chromosomes as yet show the longitudinal split preparatory for the second division, which is so often observed at this stage.

The Second Meiotic Division.—There is no resting-stage between the two divisions. In Pl. 4, fig. 31 we see

the spindles already rotating in preparation for the second division. The details of this mitosis are not so clear as those of the first, owing to the fact that the chromosomes are apt to lose their regular outlines, and to adhere together to a certain extent. A pair of metaphases are shown in Pl. 5, fig. 32. Owing to the rotating of the spindles one is seen from the pole, the other from the side. In the side view we see many of the chromosomes of the same "tetrad" shapes as those appearing in metaphase I. This has presumably arisen by a longitudinal division of the anaphasic chromosomes of the first division, with partial separation of the halves.

The shapes of the chromosomes in the anaphase II cannot be satisfactorily made out, as they always appear to stick together and separate as two solid plates in which the outlines of the individual chromosomes are only rarely plainly visible. Here and there one can often be seen to be dumb-bell-shaped, but they lose their transverse constrictions in the later anaphase (Pl. 5, fig. 33).

In spite of the obscurity of the details of this division it is plain enough that the chromosomes divide longitudinally, and not by the transverse constriction which appears in the prophase of the first division. It is by no means clear what is the meaning of this constriction, but it doubtless corresponds with the apex of the V's of the somatic or spermatogonial mitoses. Pl. 1, fig. 7 shows a daughter-plate of one of the smallest spermatogonia, in which, therefore, the chromosomes are comparatively small. An examination of the smaller elements shows that when the limbs of the V's are very short we get an appearance strongly resembling the bipartite chromosomes of the meiotic prophase.

The fact that the transverse constriction of the univalents, i.e. one of the two constrictions of the metaphase "tetrads," does not play any part in the second division will not come as a surprise to anyone who has followed the trend of recent cytology. Even the Copepod tetrads—these classical examples of reduction by means of one longitudinal and one

transverse division—have now been shown not to have the significance that has so long been attached to them. The recent works of Lerat, and especially of Matschek, have demonstrated that the transverse joint in the tetrads has no relation to the division plane of either division, the chromosomes splitting longitudinally both times. Thus in the early anaphase II the separating daughter-chromosomes are transversely jointed like those of anaphase I. The joint disappears in the late anaphase. This matter is returned to on p. 29.

Intercalation of a Resting Stage in the Meiotic Prophase.

The series of stages just described undoubtedly represents the normal course of events. Sometimes, however, the stages figured in figs. 18–23 are not immediately followed by the disappearance of the nuclear membrane and re-pairing of the chromosomes, but instead, the nucleus enters into a sort of resting stage, passing through the condition shown in Pl. 5, fig. 34 to that figured in fig. 35. The chromosomes, univalent and in the full somatic number, take up their positions equidistant from one another under the nuclear membrane, and fine threads appear joining them up. For a time they retain the appearance typical of the diakinetic chromosomes, but presently this shape is lost, and they become irregular plates of chromatin connected up with one another by numerous bridges. In most cases thirty-eight entirely separate chromosomes can be counted with ease, but often some of them show a more or less pronounced paired arrangement.

These nuclei are extremely striking objects, especially when seen with the stereoscopic eyepiece, owing to the distribution of the chromosome as thin plates close under the membrane, leaving the interior of the nucleus entirely free from any staining substance. A faintly staining nucleolus is present.

Although on looking through a number of sections a good

many of these nuclei will be found, still, they are much too rare to admit of the possibility of their being a normal stage in the prophase. I have not been able to determine whether a nucleus which has gone so far as that shown in Pl. 5, fig. 35 is capable of resuming mitosis, or whether it is destined to degenerate, but the balance of evidence is in favour of their re-entering the normal life-cycle of the spermatocyte by a disappearance of the nuclear membrane and a return to the condition shown in Pl. 3, fig. 24. In any case, the intercalation into the meiotic diakinesis of a resting or semi-resting condition of the nucleus with the somatic number of chromosomes is very remarkable.

In the spermatogonia the nucleus may also occasionally enter into a corresponding semi-resting stage in diakinesis. Part of such a nucleus (belonging to a large spermatogonium) is shown in Pl. 5, fig. 36. This cell was in a young male whose testis contained very few spermatocytes, and there were none of these in the neighbourhood of this cell, so there is no doubt of its being spermatogonial. In any case similar nuclei are frequently found, which from their position and small size must belong to the smaller generations of spermatogonia. The resemblance with the corresponding stage in the meiotic prophase is striking. As in the case of the spermatocytes, these nuclei are far too rare to be a normal stage in the spermatogonial prophase.

Except for the dissociation of the chromosomes, the semi-resting stage just described puts one in mind of the "germinal vesicle" stage of the oöcyte, and it is a very interesting fact that in one testis several spermatocytes have at this stage actually taken on the character of oöcytes. This testis came from a specimen dug out of the ground in the hibernating condition. About 11 mm. of the testis was sectioned, and within this length there are three undoubted eggs, with all the essential characteristics, both of nucleus and cytoplasm, of true ovarian eggs at an early stage of the growth period. The average diameter in μ of the cell body and nucleus in each of the three is as follows: 200 and 84,

190 and 104 (the egg figured in Pl. 5, fig. 38), 220 and 105. In Pl. 5, fig. 38 the egg is seen filling up most of the tubule in which it is situated. The cytoplasm is in the form of a very fine meshwork, the strands of which are composed of rows of very fine granules. The nucleus is full of a granular or flocculent substance, from which the chromatin strands are but indistinctly differentiated.

Besides these three eggs, the same piece of testis contains a few cells connecting them with the semi-resting spermatocytes with peripherally distributed chromosomes. One of these is shown in Pl. 5, fig 37. The diameter of the nucleus is about 10 μ more than the average spermatocyte nucleus of the same stage, being 45 μ. The cell body is 75 μ across. Not only is the size of the nucleus intermediate between that of the spermatocyte and oöcyte, but, what is more important, its structure is also. The chromosomes, in the somatic number, are distributed under the nuclear membrane in a precisely similar manner to that shown in Pl. 5, fig. 35, but in addition the cavity of the nucleus is filled with a flocculent substance such as we find in the oöcyte. For this cause the chromosomes—which are also beginning to lose their staining capacity—stand out less sharply than in the ordinary spermatocytes. The cytoplasm is still like that of the spermatocyte rather than the oöcyte, showing very little appreciable structure.

The Pre-meiotic (Spermatogonial) Divisions.

We will consider at this point the early stages of the prophase, and their significance in relation to the corresponding stages of the meiotic prophase.

The structure of the resting spermatogonial nucleus has already been described. It consists of rounded or angular blocks of chromatin connected by chromatin bridges and linin threads (Pl. 1, fig. 2). Preparation for mitosis consists in a gradual transformation of these blocks into band-like chromosomes. The first stages of this process are seen in

Pl. 1, fig. 3, in which it is seen that the large chromatin blocks are the centres for the formation of the chromosomes. These appear to grow out as continually lengthening bands, the substance of the chromatin blocks getting used up in the process (Pl. 1, figs. 3 and 4). In fig. 4 the chromosomes are fairly well defined towards one pole of the nucleus, but at the other are not nearly so far advanced. In Pl. 1, fig. 5, we have the fully formed spireme. It is distinctly formed of separate chromosomes, though the ends of these show a marked tendency to adhere together. In some cases they are connected by thin threads. It is easy to see how this condition could give rise to the apparently continuous spiremes, which have been seen in many forms.

In Pl. 1, figs. 6 and 7 are shown the equatorial plates of one of the largest and one of the smallest (earliest and latest) generations of spermatogonia. These are referred to in another connection later (pp. 30, 37). Judging from the size of the nuclei shown in Pl. 1, figs. 2, 3, 4, 5, these belong to the middle generations of spermatogonia, and would result in an equatorial plate intermediate in size between those shown in Pl. 1, figs. 6 and 7.

The localised, circumscribed area, out of which each chromosome takes its origin, is strikingly different from what we find in the early meiotic prophase. Comparing Pl. 1, figs. 3, 4, 5 with the equatorial plates, we see that when first formed each chromosome is but little longer than it will be at the metaphase—note, for instance, the very small horse-shoe-shaped element in Pl. 1, fig. 4. In the meiotic prophase, on the other hand, the chromosomes at their first appearance are enormously long and thin (leptotene threads). This difference in the early prophase is doubtless correlated with the structure of the resting nucleus. In the spermatogonia—at any rate those of the middle and later generations, to which the stages described belong—the chromosomes appear not to enter such a complete "resting" or diffused state between the mitoses as they do during the growth period of the primary spermatocyte.

The most important difference between the pre-meiotic and meiotic prophases is the entire absence in the former of any appearance of fusion of chromatin threads, such as takes place in zygonema. Although the spireme may often be observed to be longitudinally split (more often seen in the somatic than in the spermatogonal mitoses), I have never seen any suggestion of a fusion of definite widely separated threads. The really corresponding stages in the two periods seem to be (allowing for the different structures of the resting nuclei) the first resolution of the chromatin network of the spermatocyte into the leptotene threads, and the transformation of the chromatin blocks of the spermatogonia into the band-like chromosomes.

The Reduction Question.

While the majority of cytologists believe that the numerical reduction of the chromosomes is brought about by the syndesis or "conjugation" of homologous chromosomes, considerable diversity of opinion exists as to how the syndesis takes place. Some students of heredity express the opinion that the mode of conjugation is inessential, the important point being the distribution of the chromosomes to the gametes in such a way that each gamete gets one member of each pair of homologous chromosomes. However, the distribution in this manner depends upon their previous pairing, and this can only be established by observation. Now this cannot be said to have been done so long as different workers on the same group, or even on the same species, describe the syndesis as taking place at entirely different periods of the prophase, though each has observed and studied the stages described as conjugation by the others.

The various schemes of "conjugation" which have been proposed can be roughly classified into two—metasyndesis and parasyndesis (Häcker). The latter term refers to the mode of conjugation upheld in this paper for Lepidosiren, and was originally proposed by von Winiwarter and developed especially by Grégoire and A. and K. E. Schreiner. Meta-

syndesis, on the other hand, covers a wider range, and may be applied to all those other forms of conjugation in which the chromosomes are united end to end. The Copepod tetrad illustrates the simplest type of this, which is supposed by Rückert, Häcker, etc., to be produced by the junction (or non-separation during segmentation of the spireme) of pairs of chromosomes end to end, the transverse joint of the tetrad representing their point of contact, and the longitudinal one the split which runs through both chromosomes. In 1903 Montgomery attempted to show that the loops, rings, etc., of vertebrates (especially Amphibia) are of essentially the same structure, formed by the bending round of the two limbs (conjugating chromosomes) to form the rings, etc. Metaphase I thus separates whole chromosomes, which divide longitudinally in metaphase II. In 1905 Farmer and Moore independently came to the same conclusion for vertebrates (Elasmobranchs) and many other forms. Theirs is essentially a metasyndetic scheme.

If the theory of parasyndesis were established it would have a possible great significance for theories of heredity, for it allows of an extremely intimate union of the chromosomes, during which condition it is conceivable that an interchange of particles might take place (like the interchange of micronuclei between a pair of conjugating Ciliates). This would remove an often expressed difficulty of correlating Mendelian phenomena with cytological observation, namely, that the number of independently transmissible allelomorphs is often certainly much larger than the number of gametic chromosomes. In metasyndesis this intimate physiological union can play no part except as an accidental and only occasional result of a specially pronounced "second contraction." There is, for instance, no room for it in any of the older metasyndetic accounts, nor in Montgomery's scheme for Desmognathus fuscus or Farmer and Moore's Periplaneta, though it might occur in Galtonia, according to the account given by Digby, who accepts Farmer and Moore's scheme.

Bearing on the Conditions in Lepidosiren upon the Nature of the Copepod Type of Tetrad.

In regard to the simple Copepod type of tetrad, apart from the fact that Lerat describes its formation according to the parasyndetic scheme (though Matschek in his more recent work denies this), it now seems very unlikely that it is the transverse and not the longitudinal slit which represents the point of contact of the conjugants. Nevertheless, in spite of the fact, already mentioned, that he has demonstrated that the transverse joint plays no part in either division, Matschek still maintains that it is an indication of an end-to-end, but permanent, junction of pairs of chromosomes. Häcker has quite recently (1910) re-stated his views regarding the meaning of the Copepod maturation divisions, in accordance with the latest work on this group. He thinks that the numerical reduction is brought about by the end-to-end junction of pairs of chromosomes, of which the transverse joint is the outward sign. Since the pairs of chromosomes are divided in each division in a plane running longitudinally through both chromosomes, it follows that each gametic nucleus gets the full number of chromosomes, joined however in pairs to form the apparently reduced number. This pairing is a permanent one, and the fusion of the conjugants is presently complete, as shown by the fact that the transverse joint gradually disappears.

If it be granted that the transverse constriction of the quadripartite chromosomes of the metaphase of the first meiotic division in Lepidosiren (i. e. the constriction parallel with the spindle axis, which is the same thing as the transverse constriction of each diakinetic univalent) is homologous with the transverse joint of the Copepod tetrad, it would be impossible to agree with Häcker's scheme. There seems no reason to doubt the homology. The resemblance is close and the behaviour identical, i. e. appearing in diakinesis and persisting through the maturation divisions without acting

as the division plane in either, and gradually disappearing in anaphase II. Now in Lepidosiren it seems certain (apart from the correctness or otherwise of my interpretation of the previous stages) that the constriction cannot possibly be the sign of an end-to-end junction of the chromosomes, seeing that it is present in the diakinetic univalents. Probably no one will question that these are univalent and not each composed of two half chromosomes joined end-to-end. It will be remembered that they are widely separated, and in the later diakinesis become distributed equi-distantly from one another under the nuclear membrane. In this state they may even enter into a resting condition. A comparison of the meiotic and pre-meiotic nuclei in this condition (Pl. 5, figs. 35 and 36) will probably complete the proof if any be needed. It would be difficult to hold that in the one case (fig. 36) each chromatin mass has the value of a whole chromosome, and in the other (fig. 35) the value of two half chromosomes joined together.

Another difficulty in the way of considering the transverse constriction as an indication of an end-to-end junction is its almost certain homology with the apices of the V's of the spermatogonial or somatic chromosomes. Attention is drawn to this on p. 22. In addition to what is said there, it should be noted that in the smaller spermatogonia the limbs of the V's often seem very loosely connected at the apex (see fig. 7, 1–6), so that at first glance they look almost like separate chromosomes. This reminds us of the apparently nearly complete separation of the halves of the tetrads by the transverse joint so often seen in other forms. In the larger spermatogonial and somatic divisions the angle between the limbs of the V's is not nearly so sharp—in fact the chromosomes often show no tendency to bend at one spot more than another. This may account for the unequal lengths of the limbs in some of the chromosomes, though, as may be seen by reference to the figures, the transverse constrictions do sometimes divide the chromosomes into unequal portions even in the meiotic divisions, though the inequality is naturally

less striking than in the case of the long, thin, pre-meiotic chromosomes.

Similar considerations may be applied to all those forms with simple tetrads divided by a longitudinal and a transverse joint, in which the presence of the transverse joint (i.e. the one parallel with the spindle axis in the metaphase) is taken as evidence of an end-to-end junction of the chromosomes.

A recent work purporting to describe the origin of the tetrads by a simple end-to-end conjugation with subsequent longitudinal splitting is that of Gross, who has re-described the spermatogenesis of Pyrrhocoris. Now an examination of his text and figures makes it appear extremely probable that the diakinetic stages are really similar to those of Lepidosiren. The difficulties which lie in the way of a correct interpretation in Pyrrhocoris, and the comparative certainty with which these stages can be elucidated in Lepidosiren is my excuse for attempting to criticise Gross's account. According to this author the first synizesis loosens into long chromosomes, of which the number could not be exactly determined, but which were certainly more than the reduced number. Now his figures 17 and 18 look very like nuclei containing the reduced number of gemini of which the members are well separated, though the pairs are still mostly distinguishable. As he gives no figures or description of the stages before synizesis, which he says follows almost directly after the last spermatogonial division, it is impossible to form any opinion as to how these gemini have been produced. The separation of the gemini (supposing my interpretation to be correct) becomes successively more and more complete in Gross' figs. 19, 20, 21, till they are as well separated as in Lepidosiren. After the peculiar temporary disintegration of the chromosomes, a second synizesis sets in. As the chromosomes enter into this contraction as apparent monads and emerge dumb-bell-shaped, Gross assumes that during this stage they have conjugated in pairs. Gradually in successive stages we find more and more quadripartite rings and fewer and fewer dumb-bells. Gross interprets the tetrad

rings as formed by splitting of the dyads, but his figures show such a close resemblance to the secondary pairing in Lepidosiren that it appears to me impossible to escape from the conclusion that in Pyrrhocoris the rings are formed in the same way, that is, by a pairing of the bipartite univalents, and that the change from the monad to the dyad appearance during the second synizesis is not due to conjugation, but to the development of a transverse constriction in each separate univalent as we saw so diagrammatically in Lepidosiren. Unfortunately Gross does not give us the only information which could settle this point. He makes no counts of the chromosomes until after the stage where ring formation is complete, by which time, of course, the reduced number is present. In this respect Henking's account is more complete than Gross's, and seems fully to confirm the interpretation which I have ventured to suggest. Henking states that in nuclei containing a mixture of rings and half rings, the total number of chromosomes, counting rings as two and half rings as one, adds up to twenty-four (the somatic number).

Reality of Parasyndesis.

The debatable stages of the meiotic prophases in which parasyndesis and its associated phenomena occur—leptonema, zygonema, strepsinema—have been dealt with by many experienced cytologists, such as Montgomery, Farmer and Moore, Grégoire, A. and K. E. Schreiner, Janssens, Meves, Fick, Goldschmidt, Häcker and many others, in whose works are to be found full discussions of the evidence for and against.

The establishment of parasyndesis, with subsequent separation of the homologous chromosomes in the first meiotic division, may be said to rest on two main foundations. Firstly, that the leptotene threads have each the value of a whole chromosome, and that they do not represent the temporarily separated daughter halves of a precociously split spireme.

In such forms as Lepidosiren, where the resting nucleus is first resolved into a tangle of leptotene threads, which can be traced for long distances and are found to run across one another in all directions, it does not seem possible to homologise them with the daughter halves of a double spireme, and yet there seems no room for doubt that these are the threads which fuse in pairs later in zygonema. It is true that by the time the zygonema is fairly far advanced we do get appearances not unlike what may occasionally (but not regularly) be found in the condensation of a somatic chromosome, as Digby has pointed out in a recent paper dealing with this question. To make this, however, a basis of comparison of somatic with meiotic prophases, to the end of denying the significance of parasyndesis, is to take no account of the preceding stage of leptonema.

The second foundation on which the establishment of parasyndesis rests, is that the spaces between the fusing leptotene threads (which become the longitudinal split visible here and there in the pachytene threads or thick spireme) is the space which reopens in strepsinema to form the space separating the two branches of the gemini, whether these are arranged in parallel bars, rings, crosses or other figures, and is the split which becomes effective in the first heterotype division. This is in accordance with the opinion of most cytologists, in opposition to the belief of Montgomery, Farmer and Moore, and their followers, who hold that the rings, etc., of metaphase I are formed by the horse-shoe-shaped chromosomes of the bouquet stage (each bivalent being formed of two chromosomes united by one of their ends at the apex of the horse-shoe) bending over their free ends in the "second contraction" to form the closed rings, figures-of-eight, or other forms met with in diakinesis. According to this view the longitudinal split visible in the early spireme is in preparation for the second division. As is well known, the majority of both schools are agreed as to the composition and further fate of the gemini once formed, i.e. that each is composed of two approximated somatic

chromosomes, which separate from each other in metaphase I.

As was brought out in the descriptive part, the onset of strepsinema is obscured in Lepidosiren by the synchronous beginning of synizesis, so that this stage is the least clear of any in this animal. Consequently the direct evidence to be gained from it, while all in favour of the most usually accepted view that the rings are formed by the re-opening of the longitudinal split temporarily obliterated in the pachytene stage, is, it must be admitted, of less value than that to be gained from other forms in which this stage is clearer.

Significance of the Temporary Complete Separation of the Ex-conjugants in Diakinesis.

The complete separation of the ex-conjugants, which is begun in synizesis and completed in late diakinesis, is one of the most striking features of the maturation processes in Lepidosiren, and a full account has been given of it in the descriptive part. Nevertheless, it is only an extreme example of the very loose connection between the branches of the gemini so often observed in diakinesis. It is only necessary to glance through the plates of a few works on spermatogenesis, or still more of oögenesis, to convince oneself of this.

In many forms a diakinetic end-to-end pairing has been described, closely similar to the second pairing in Lepidosiren, and has been taken for the real conjugation. The case of Lepidosiren makes it evident, however, that the mere fact that the chromosomes are present in the somatic number in diakinesis and then unite end to end in pairs is no proof that the parasyndetic scheme does not apply to them. As examples of forms with a diakinetic pairing like that of Lepidosiren may be mentioned Lumbricus (Calkins), Caloptenus (Wilcox), Œnothera (Gates, Davis; in this genus the pairing is often a very loose one), and possibly Ophryo-

trocha (Korschelt's account; in this case also the pairing is extremely loose).

The separation and second pairing of the ex-conjugants gives indirect support to the view that the first pairing was by parasyndesis. For if not—if, that is to say, the reduced number of chromosomes in the bouquet stage has been brought about by an end-to-end junction of the chromosomes —it seems extraordinary that they should separate, only to come together again in exactly the same way. On the other hand, if the original conjugation were by parasyndesis a possible explanation presents itself, and this is connected with the important question of the function of chromosome conjugation. It was suggested by the Schreiners in an interesting paper that this function is a double one. The intimate fusion during the prophase (parasyndesis) is to be considered as a physiological process of a "rejuvenescing" nature, in which interchange of substance takes place. After conjugation, that is, in strepsinema, the ex-conjugants separate physiologically, but remain approximated or even united by their ends or elsewhere in order to ensure the characteristic distribution of the chromosomes in the reduction division. Thus the separation of homologous chromosomes to different gametes, often considered the whole object of conjugation, is only one of two independent functions. The case of Lepidosiren supports this view. If the whole object of conjugation were to bring about the separation of homologous chromosomes in metaphase I, it would appear inconceivable why the chromosomes, having once paired (and that they are of the reduced number in the bouquet stage is a matter of observation, independent of any view as to how the pairing has taken place), should separate only to pair again.

The fact that in certain parthenogenetic eggs (Cladocera, Kühn) appearances of parasyndesis are found, although in the equatorial plate of the maturation division the somatic number of chromosomes appears, has been taken by some as a final refutation of the theory of parasyndesis, e. g., Goldschmidt says: ". . . zum Schlusse noch eines—auch bei partheno-

genetischen Eiern parthenogenetischer Generationen findet man genau gleichen Bilder, die sonst als parallele Konjugation homologen Chromosomen gedeutet worden (s. die Arbeit von Kühn in diesem Heft). Wo bleibt da die Theorie?" Kühn, however, himself suggests that there may have been a parasyndesis and subsequent separation of the conjugants. The case of Lepidosiren greatly strengthens this possibility. By missing out the second diakinetic pairing and one (the first) of the maturation divisions, a condition similar to that found in the Cladocera would be arrived at—and that the condition of parthenogenesis has been derived somehow from the sexual one is, of course, unquestionable.

Strasburger has made a series of observations on the cytology of plant apogamy (i. e. parthenogenesis without reduction of chromosomes) which are in accord with these views. He finds that in Marsilia Drummondii there is a synaptic contraction as in allied sexual forms, in which conjugation presumably takes place. The first peculiarity appears in diakinesis, in that some of the nuclei are diploid instead of the expected haploid, and some intermediate. He interprets the diploid condition as due to separation of the ex-conjugants, which is also borne out by the comparative sizes of the diakinetic chromosomes in haploid and diploid nuclei. In Elatostema acuminata there is a synaptic contraction, but this resolves itself into a resting network again, and then ordinary somatic division follows. In E. sessile and Wikstroemia indica he finds no sign of synaptic contraction in the prophase, and takes this as proof that there is no conjugation of chromosomes.

If it be permitted to speculate from the facts just recorded, it is tempting to look upon the relation of parthenogenesis to chromosome conjugation as follows.

In parthenogenetic reproduction the physiological conjugation of the chromosomes (parasyndesis) is retained (Cladocera, Marsilia) but the ex-conjugants separate entirely as in Lepidosiren. Unlike what happens in this animal, however, they do not pair again, and in correlation

with this the first maturation division is omitted, and consequently each egg gets the somatic number of chromosomes. Should this be the case, it emphasises strongly the double function of the conjugation of the chromosomes. If Strasburger is right in interpreting the absence of a synaptic contraction in Elatostema sessile and Wikstroemia as indicating an absence of chromosome conjugation it shows of course that its periodical occurrence is not essential to every form, but this does not destroy its significance any more than the occurrence of a few parthenogenetic species does away with all the meaning of conjugation of gametes.

Pairing of Chromosomes outside the Meiotic Period.

In somatic and spermatogonial equatorial plates it can be seen at first glance that the arrangement of chromosomes is not haphazard. This was noticed by Murray in his paper on the somatic mitoses in Lepidosiren (in various embryonic tissues). He says: "Die Grössenunterschiede der Chromosomen kommen auch in anderen Weise zum Ausdruck, indem sie die Anordnung der Kernelemente in der Tochterplatte zu bedingen scheinen. In der Mitte . . . liegen die kleinsten Chromosomen . . . Die grössten Elemente liegen ganz peripher . . . Die Mittelgrössen chromosomen nehmen eine Zwischenstellung ein."

These observations of Murray's I can fully confirm and extend them to the spermatogonial mitoses. In the metaphase of both somatic and spermatogonial divisions the chromosomes are generally arranged in a hollow ring, so that the small chromosomes are not situated to the inside of the larger ones, but we find the latter grouped towards one side and the former towards the other side of the equatorial plate (Pl. 1, fig. 6). In the anaphase the ring shape is destroyed and the distribution described by Murray appears (Pl. 1, fig. 7).

In the large spermatogonia the exact delimitations of the chromosomes are not always easy to determine owing to their great length, which causes them to overlap one another

(Pl. 1, fig. 6), but the general grouping of longer chromosomes at one side of the equatorial plate and smaller ones at the other is evident. Much clearer figures are obtained in the case of the smaller spermatogonia with their comparatively short chromosomes (Pl. 1, fig. 7).

Since this characteristic grouping is present not only in the metaphase, but also in the anaphase, it must have the result that homologous chromosomes, or rather chromosomic areas, are near one another in the resting nucleus.

Besides this general arrangement of the large and small chromosomes, an examination of the figures gives one a very distinct impression that there is a tendency for pairs of chromosomes of equal size to approximate to one another. This is clearest in the small spermatogonia, both because of the greater simplicity of the figures, and also because the size differences seem to be emphasised in them. In Pl. 1, fig. 7, the large V's are closely approximated, and several other pairs can be made out.

This pairing of corresponding chromosomes outside the meiotic phase has been frequently noticed and taken to be a sign of a mutual attraction, which is brought to a head in the meiotic prophase. Montgomery (1904) described it in the spermatogonia of various species of Amphibia, A. and K. E. Schreiner in Spinax spermatogonia, and Müller in the somatic mitoses of Yucca. In this form the paired arrangement persists in the late telophase and reappears in the earliest prophase, and consequently it is a fair presumption that it is maintained throughout the resting nucleus. The most convincing case of all is that of Culex sp. (Stevens), in which the six chromosomes always appear in three obvious pairs in the pro- and meta-phases of the oögonial divisions.

The tendency of the smaller chromosomes to lie in the centre and the larger ones on the periphery can be seen in almost any polar view of a mitosis with marked size differences among the chromosomes.

Summary.

The different generations of cells composing the germinal epithelium resemble those often described in other forms, especially in the Amphibia. Very little arrangement of the different generations in different parts of the testis could be observed.

The somatic number of chromosomes is thirty-eight. One pair of these is conspicuously larger than the rest.

The reduced number of chromosomes in the bouquet stage appears to be arrived at by a parallel conjugation in the early prophase according to von Winiwarter's scheme.

In strepsinema, which synchronises with the onset of synizesis, the conjugants separate except at their ends, to form very long-drawn-out rings.

During synizesis and diakinesis the rings break into their constituents, and the somatic number of univalent chromosomes is again obtained, the "homologous" chromosomes being often widely separated from each other.

During diakinesis each univalent becomes divided by a transverse constriction, which probably corresponds with the apices of the V's of the pre-meiotic chromosomes, and also with the transverse division of the Copepod type of tetrad, which cannot therefore be taken as indicating the point of junction of two chromosomes united end to end. As in Copepods, the transverse constriction is not the division plane in either mitosis, but disappears during anaphase II.

After the dissolution of the nuclear membrane "homologous" chromosomes are seen to approach each other, and join together a second time to form the rings or modifications of them found in metaphase I.

The first maturation division separates entire "homologous" chromosomes.

There is no resting stage between the two divisions. In the second division the chromosomes divide longitudinally, forming "tetrads," etc., very like those of metaphase I.

A resting or semi-resting stage may be intercalated into

the spermatocyte diakinesis. In this condition the chromosomes, in the somatic number, are distributed round the periphery of the nucleus just under the nuclear membrane. In advanced stages they lose their regular shapes and become connected by numerous anastomoses. In one testis several spermatocytes in this stage have taken on all the characters of oöcytes.

The spermatogonial prophases are of a very simple nature, and show no sign of anything comparable to the stage of zygonema.

In the spermatogonial and also in the somatic nuclei the chromosomes are arranged in a definite plan, the smaller and larger ones being grouped together. Within the main groups there is also evidence of a tendency for chromosomes of equal size to be next to each other.

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EXPLANATION OF PLATES 1–5,

Illustrating Dr. W. E. Agar's Memoir on "The Spermatogenesis of Lepidosiren paradoxa."

[All figures were drawn with the Abbé camera, and except figs. 37 and 38, under a magnification of 2100 diameters (Leitz $\frac{1}{12}$ oil imm., Zeiss 12 comp.) In most cases the details were confirmed with the help of Zeiss's stereoscopic eyepiece. Final magnification of figures, as reproduced, 1400 diameters. In no case, except fig. 38, has any attempt been made to show the structure of the cytoplasm. In every case the most superficial chromatin is most darkly shaded, the deeper parts being shown paler.]

LETTERING.

b. Bivalent. *l*. A member of the large pair of chromosomes. *n*. Nucleolus. *p*. Ex-conjugants in the act of pairing for the second time. *s*. Conjugants separating. *u*. Univalent.

Fig. 1.—Resting spermatogonium from one of the earlier generations, showing diffuse substance filling the nucleus and largely concealing the chromatin. Corrosive-acetic.

Fig. 2.—Spermatogonium from one of the earlier generations of the period of active multiplication. Flemming.

Fig. 3.—Spermatogonium, very early prophase. Chromatin blocks beginning to break up and spread out into bands. Corrosive-acetic.

Fig. 4.—Spermatogonium, later prophase. The individual chromosomes are in many cases clearly definable. Corrosive-acetic.

Fig. 5.—Spermatogonium, fully formed spireme stage. The "spireme," however, is plainly not continuous. Corrosive-acetic.

Fig. 6.—Equatorial plate of one of the earlier generations of spermatogonia. Corrosive-acetic.

Fig. 7.—Equatorial plate of one of the later generations of spermatogonia. Nos. 1–6 are each a single sharply bent chromosome. Corrosive-acetic.

[In figs. 8–15 only the chromatin lying close under the nuclear membrane is shown.]

Fig. 8.—Primary spermatocyte at end of growth period. Less than half the nucleus is contained in the section, so that its full diameter is not shown. Flemming.

Fig. 9.—Leptotene stage. Flemming.

Figs. 10–12.—Lepto-zygotene stage. In each case only the top of the nucleus is shown. Fig. 12, a polar view. All Flemming.

Fig. 13.—Zygo-pachytene stage. Chromosomes arranged in bouquet form. Flemming.

Fig. 14.—Pachytene stage, polar view. Corrosive-acetic.

Fig. 15.—An unusually large pachytene nucleus. The reduced number of loops is present here (see p. 13). Corrosive-acetic.

Fig. 16.—Polar view of strepsinema (diplonema). Synizesis is setting in. Most of the pachytene threads have split apart along their whole lengths (so far as can be seen) with the exception of their extreme ends, which remain united, thus forming long rings. In some places, however, the process of splitting can still be seen. One ring is cut through by the razor, showing two free ends. Flemming.

Fig. 17.—Synizesis further advanced. Flemming.

Figs. 18–23.—Stages showing breaking up of synizesis, shortening and thickening of chromosomes, complete separation of the ex-conjugants and development of the transverse constriction in the univalents. Described on pp. 16–18. All nuclei, except fig. 21, untouched by the razor. All in 35–40 μ celloidin sections, mounted without dissolving out the celloidin. Fig. 20, two adjacent nuclei. In fig. 18 one of the elements is drawn separately. All corrosive-acetic.

Fig. 24.—Immediately after disappearance of the nuclear membrane. Thirty-eight free, transversely constricted, univalent chromosomes. The nucleus is cut in two sections, both of which are shown. Flemming, paraffin section.

Figs. 25–28.—Stages in the second pairing of the chromosomes.

Fig. 25.—Twenty-six unpaired univalents, six bivalents, in three of which (*b*1–*b*3) pairing is complete; the other three (*b*4–*b*6) have united by one end only. Nucleus intact, in 40 μ celloidin section. Corrosive-acetic.

Fig. 26.—Two views of the same nucleus, obtained by turning over the section (mounted between two coverslips) and drawing from both sides. In each figure all the chromosomes are shown, but those in the upper optical section are shaded, and those in the lower half shown only in outline. *b*, a complete bivalent; 1 and 3, 5 and 6, 7 and 8, 9 and 10 pairing chromosomes; 2 and 4 are probably the large chromosomes. Untouched nucleus in 40 μ celloidin section. Corrosive-acetic.

Fig. 27.—Only two univalents left (*u*1, *u*2), fifteen completed bivalents in the form of rings, and three (one of them *l*, the large pair) united by one end only. 40 μ celloidin. Flemming.

Fig. 28.—Pairing complete. Some of the bivalents, to show characteristic shapes and size differences. Flemming.

Fig. 29.—Metaphase I, polar view. Corrosive-acetic.

Fig. 30.—Early anaphase I. Flemming.

Fig. 31.—Late anaphase I. Spindles rotating for second division. Flemming.

Fig. 32.—A pair of metaphases II. Corrosive-acetic.

Fig. 33.—Anaphase II. Flemming.

Fig. 34.—A nucleus which, after passing through stages of the meiotic prophase as far as the dissociation of the ex-conjugants, is entering into a resting stage instead of proceeding to mitosis. The nucleus is cut in two sections, both of which are shown. Thirty-seven chromosomes are seen. In addition there was one more lying outside the nucleus, having evidently been displaced by the razor. 40 μ celloidin section. Corrosive-acetic.

Fig. 35.—"Resting" stage more complete. Thirty-eight chromosomes present. This nucleus is just shaved at both surfaces, and two chromosomes, one at the top and one at the bottom, have been added from the preceding and succeeding sections. 30 μ celloidin section. Corrosive-acetic.

Fig. 36.—Part of a similar "resting" nucleus from a spermatogonial prophase. Corrosive-acetic.

Fig. 37.—Transition of spermatocyte like that shown in fig. 35 into an oöcyte-like condition. Nucleus not intact, several chromosomes being in the next section. Zeiss 3 mm. apochr., 12 comp. Corrosive-acetic. 40 μ celloidin.

Fig. 38.—Oöcyte characters fully assumed. *spm*. Spermatogonium. *spc*. Spermatocytes I. *ov*. Oöcyte. Zeiss D. 2. Corrosive-acetic. 40 μ celloidin.